The Assimilation of Nitrogen by certain Nitrogen-fixing Bacteria in the Soil.

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In a communication on "Some Effects of Nitrogen-fixing Bacteria on the Growth of Non-Leguminous Plants,"* it was pointed out that *Azotobacter* and *Pseudomonas* obtained from the root tubercles of Cycas when grown together fix more nitrogen per unit of carbohydrate than the combined amount of nitrogen when each is grown separately. In order to determine if this is true for a mixed culture of *Azotobacter* and *Pseudomonas* obtained from ordinary soil and leguminous nodules respectively, pure cultures of these organisms were obtained, *Azotobacter chroococcum* from garden soil and *Pseudomonas radicicola* from bean and clover nodules, by the method already described.* Erlenmeyer flasks containing a culture solution, consisting of maltose 0·5 gramme, mannite 0·5 gramme, monobasic potassium phosphate 0·1 gramme, magnesium sulphate 0·02 gramme, in 100 c.c. distilled water and rendered neutral by sodium hydrate, were inoculated with 1 c.c. of pure cultures per 100 c.c. of culture solution (the controls being autoclaved to kill the bacteria present), and incubated at 24° C. for 10 days. Nitrogen determinations of the contents of the flasks gave the following averages :—

Control.....	0·53 milligramme N in 100 c.c. per unit of carbohydrate.
Azotobacter alone	2·19 milligrammes N in 100 c.c. per unit of carbohydrate.
Pseudomonas alone	2·30 milligrammes N in 100 c.c. per unit of carbohydrate.
Pseudomonas + *Azotobacter* ...	4·51 milligrammes N in 100 c.c. per unit of carbohydrate.

Gerlach,† Lipman,‡ and others have described experiments showing that pure cultures of *Azotobacter* and *Pseudomonas* respectively have little or no power to increase the store of soil nitrogen when added directly to the soil. A probable explanation of these negative results may be found in the

* 'Proc. Roy. Soc.,' B, vol. 81, pp. 287–289.

† 'Centralbl. f. Bakt. Abt. II.,' vol. 8, 1902.

‡ 'Reports New Jersey Expt. St.,' 1904—1907.

different conditions for growth and development in ordinary soil and in a culture solution.

In order to induce the pure cultures to accommodate themselves to soil conditions, the following method was employed:—Some ordinary garden soil was treated with lime and sterilised in the autoclave. This was then thoroughly wetted with pure cultures of *Azotobacter* and *Pseudomonas*, and incubated at a temperature of 24° C. for 21 days. The organisms multiplied rapidly and spread through the soil, and adapted themselves to the special chemical and biological conditions to which they were subjected. Five grammes of this infected soil were mixed in 100 c.c. of water, to which 1 gramme of glucose had been added, and incubated for 24 hours. To test the effect of this culture solution in fixing nitrogen in the soil a series of five earthenware plant dishes were each filled with 5 oz. of rich garden soil which had been air dried and passed through a fine sieve to remove all stones, twigs, etc. Three of the dishes were limed—$\frac{1}{4}$ oz. of lime to each dish. The dishes were treated as follows:—

1. Watered with 50 c.c. distilled water.
2. Watered with 50 c.c. culture solution which had been autoclaved to kill the bacteria.
3. Watered with 50 c.c. living culture solution.
4. Watered with 50 c.c. distilled water.
5. Watered with 50 c.c. living culture solution.

The dishes were then placed in the incubator and incubated at 24° C. for 10 days, the soil being stirred with a sterile glass rod each day for aeration, and each dish receiving 50 c.c. of distilled water on the third, fifth, and eighth days of the experiment, to supply the loss of moisture due to evaporation.

The nitrogen determinations of the contents of the dishes gave the following results:—

1. Limed soil + distilled water	324 milligrammes N per gramme soil.
2. Limed soil + autoclaved culture	330 milligrammes N per gramme soil.
3. Limed soil + living culture	359 milligrammes N per gramme soil.
4. Unlimed soil + distilled water	327 milligrammes N per gramme soil.
5. Unlimed soil + living culture	352 milligrammes N per gramme soil.

Thus the mixed culture of *Azotobacter* and *Pseudomonas* gave an increase of 35 milligrammes of nitrogen on the limed soil, and an increase of 25 milligrammes of nitrogen on the unlimed soil.

This gain of nitrogen was not due to any material present in the culture solution, for the autoclaved culture solution shows a gain of 6 milligrammes of nitrogen only, derived chiefly from the dead bacteria in the solution.

Taking an acre of soil 4 inches deep as weighing about 1,000,000 lbs., a gain of 35 milligrammes of nitrogen per 100 grammes would represent an increase of nearly 350 lbs. of nitrogen per acre.

That the nitrogen fixed by this mixed culture of bacteria in the soil is readily assimilated by plants is shown by a number of experiments now in progress, full details of which will be described in a future communication.

On the Structure, Development, and Morphological Interpretation of the Pineal Organs and Adjacent Parts of the Brain in the Tuatara (Sphenodon punctatus).

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(Abstract.)

The memoir of which an abstract is here given contains a detailed account of the pineal organs and associated parts of the brain in Sphenodon, from the morphological, histological, and embryological points of view, accompanied by numerous illustrations, and may be regarded as a continuation and amplification of my earlier work on the subject.

The material upon which my results are based consisted partly of a number of adult living Tuataras presented to me by the New Zealand Government, the cost of transmission of which to England was defrayed by a grant from the Government Grant Committee, and partly of specimens (chiefly embryos) preserved by myself while in New Zealand. I defer the expression of my thanks to the numerous friends who have helped me in the work until the publication of the complete memoir.

As I have already pointed out in my work on the intracranial vascular system,* there is in Sphenodon a very extensive subdural cavity between the brain and the cranial wall, and advantage was taken of this fact to fix the

* 'Phil. Trans.,' B, 1909.